Director, IVF Program, Division of Reproductive Endocrinology & Infertility

Date: January 17, 2006

To: All IVF candidates

From: Chief, Reproductive Endocrinology & Infertility

RE: Criteria for IVF program

Based on published data and resource availability the following are the criteria to be enrolled in the IVF program at TAMC. There are no exceptions.

1. **The female patient must be no older than 41 years of age** at the time of the IVF stimulation start. **Upon turning 42, no further IVF cycles will be preformed**. The literature demonstrates poor IVF success in patients over 40 years.
2. **The day 3 FSH in our lab must be less than 12 for all patients.** The literature supports these values and age ranges. Outside lab values may need to be repeated if they are slightly elevated in our lab.
3. **Weight limit of 250 pounds**. This limit is secondary to equipment used at the outside laboratory.
4. The female patient must have a uterus and at least one ovary. She also must be in good health and, if she is under the care of a doctor for a medical condition, a note from her physician is required stating that pregnancy would not worsen her condition.
5. If the male or the female patient has a terminal or life threatening disease then they are not candidates for IVF.
6. The source of the sperm must be the husband of the female patient or an anonymous donor from a reputable sperm bank in the U.S.
7. HIV status must be negative for both the patient and the spouse.
8. Couples currently separated or undergoing marital counseling are not candidates.
9. A consult from your physician is required and must include a complete and up to date history and physical exam.
10. Patients will be assigned to the IVF waiting list on a first come basis. No priority will be given to age, rank, diagnosis, or other circumstances such as TDY or PCS dates.
11. Those patients completing an IVF cycle without achieving pregnancy will be placed at the end of the waiting list if they desire to attempt a subsequent IVF cycle.

Chief, Reproductive Endocrinology & Infertility

Assisted Reproductive Technology Manual

Tripler Army Medical Center

Assisted Reproductive Technology Manual

Acknowledgements:

This manual is modified from a similar document provided by the South Texas Fertility Center. We are grateful to Robert G. Brzyski, M.D., Ph.D. for allowing us to modify the document for our purposes.

Charts and Graphs are from the CDC 2003 Report.

The patient information sheets are provided by the American Society of Reproductive Medicine.

Fertility Facts

What is an Infertility Specialist?

The medical specialists that treat patients with infertility are known professionally as Reproductive Endocrinologists. Training in Reproductive Endocrinology requires a medical school degree. The physician must then complete a four-year residency in Obstetrics and Gynecology (OB/GYN), during which she or he receives broad training in general Obstetrics and Gynecology. The final course of training is a three-year fellowship in Reproductive Endocrinology. Fellowship training focuses on the diagnosis and treatment of infertility and related disorders. This training includes experience in microsurgery, laparoscopic and hysteroscopic surgery, *in vitro* fertilization-embryo transfer, sonography, and ovulation induction. In addition, the physician spends a significant amount of time performing clinical and/or laboratory research.

Assisted Reproductive Technologies

Assisted Reproductive Technologies (ART) include *in vitro* fertilization-embryo transfer (IVF-ET), gamete intrafallopian transfer (GIFT), zygote intrafallopian transfer (ZIFT), tubal embryo transfer (TET), and frozen embryo transfer (FET). Although ART has helped many people overcome their infertility, they are not the answer for every infertile couple. Most of the time we use ART only when less complex and less expensive methods of treatment have failed. However, in certain circumstances (such as advanced age or severe male factor) we may recommend ART as first-line therapy.

In vitro fertilization, GIFT, ZIFT, and TET are very similar procedures although there are a few significant differences. During IVF-ET, ZIFT, and TET, the oocytes (eggs) and sperm are combined in a culture dish in the laboratory. Fertilization and very early embryo development occur outside the body, rather than in the fallopian tube. Once early embryo development is recognized, the embryos are transferred either into the uterus (IVF-ET) or the fallopian tube (ZIFT, TET). Since we have seen no significant difference in success rates, we usually perform IVF-ET because it is less expensive and doesn't require laparoscopy and general anesthesia. In addition, IVF-ET is the only procedure available for women with damaged fallopian tubes.

GIFT differs from the other procedures in that sperm and oocytes are transferred into the fallopian tubes immediately after oocyte retrieval. Fertilization thus occurs in the body, rather than in the laboratory. GIFT originally was thought to represent a breakthrough in infertility therapy. National ART statistics suggest that success rates are higher with GIFT than IVF-ET. However, many investigators have concluded that GIFT does not increase the likelihood of conception compared to other ART procedures, and that the statistics reflect differences in laboratory expertise or in the kinds of patients treated with GIFT versus IVF-ET. In addition, GIFT does not allow for confirmation of successful fertilization if the procedure does not produce a pregnancy.

For these reasons, the vast majority of ART procedures performed are IVF-ET.

Couples who are considering ART should realize that it is an intensely emotional, physically arduous, and expensive procedure. Most couples find it difficult to consider the chances for success realistically without dampening the drive that allows them to undertake these procedures. Above all, couples should explore plans for the future, whether or not their attempts at ART are successful.

Candidates for ART

Every patient should have completed a basic infertility evaluation. Because of the physical, emotional, and financial demands of ART, these procedures generally are used in patients who have tried less complex and less expensive methods of correcting their infertility. The majority of patients in ART programs suffer from tubal factor, male factor, or unexplained infertility. ART candidates must be under 42 years of age and must have:

- No evidence of premature menopause
- At least one accessible ovary, and
- A normal uterus

Menopause and ovarian function are irrelevant for candidates using donor eggs. Although we do not offer donor egg ART at Tripler, donor egg recipients should be under 50 years of age and have a normal uterus. All ART candidates should be in good health and have no medical conditions that would pose a serious health risk to themselves or the child they would carry.

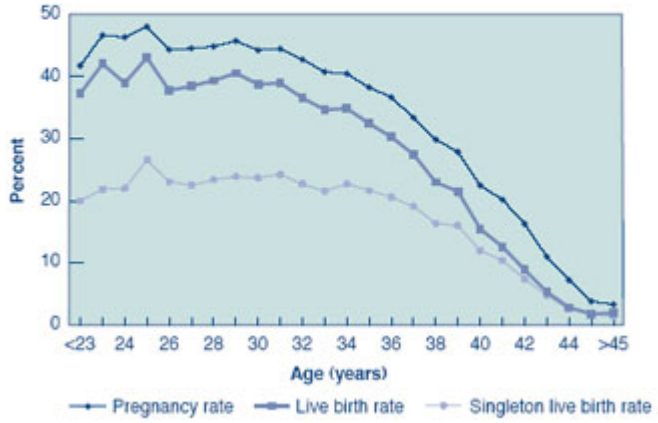
Participation in an ART program can be stressful and emotional. We encourage couples considering or pursuing ART therapy to attend group support sessions such as those offered by RESOLVE, a national infertility support group which has an active local chapter in Honolulu. We can also refer you to private counselors for individualized care.

Success Rates

One of the first questions that couples ask is "What is our chance for success?" The initial hope of achieving a pregnancy by ART is often dampened by the answer to this question. In 2003, in our program, liveborn infants occurred in 54% of cases in which women had embryos transferred to the uterus. The 2003 nationwide "take home baby rate," was 34%. We believe that the delivery rate or "take home baby rate" is the only real measure of success. Patients should be aware, however, that some clinics define "success" as any positive pregnancy test, or any pregnancy, even if miscarried or ectopic. These "successes" are irrelevant to patients desiring a baby.

Success varies with many factors. One very important factor is the age of the woman. Over age 37, ART success rates decline dramatically.

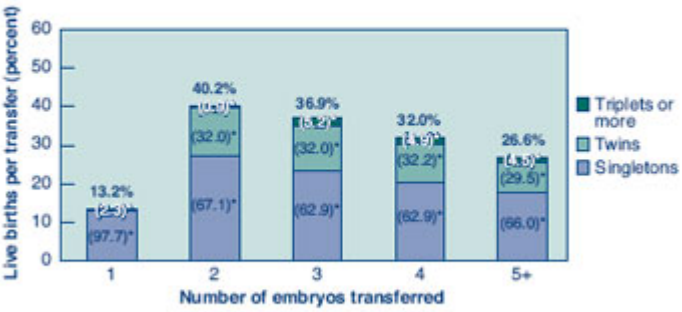
Figure 10
Pregnancy Rates, Live Birth Rates, and Singleton Live Birth Rates for ART Cycles Using Fresh Nondonor Eggs or Embryos, by Age of Woman,* 2003



* For consistency, all rates are based on cycles started.

Another factor that affects ART success is the number of embryos that are transferred. According to 2003 national statistics, about 13% of cycles in which one embryo was transferred resulted in a live birth; with two embryos, the success rate increased to 40% but with an increased risk of twin and triplet pregnancies.

Figure 28
Live Births per Transfer and Percentages of Multiple-Infant Births for ART Cycles Using Fresh Nondonor Eggs or Embryos, by Number of Embryos Transferred, 2003



* Percentages of live births that were singletons, twins, and triplets or more are in parentheses. Note: In rare cases a single embryo may divide and thus produce twins. For this reason, a small percentage of twins resulted from a single embryo transfer, and a small percentage of triplets resulted when two embryos were transferred.

Presently, the collection of oocytes, fertilization, and early embryo growth, are accomplished with a high degree of efficiency. The major hurdles to success are implantation after embryo

transfer and early pregnancy loss. The rate of early pregnancy loss is slightly higher with ART compared to spontaneous conception. The risk of early pregnancy loss increases with age of the female partner. There is, however, no evidence that the risk of birth defects or chromosome abnormalities (such as Down's syndrome) is any different with ART. Pregnancy complications tend to be higher with ART pregnancies, primarily because of the much higher rate of multiple pregnancies. Twins occur in about 31% of ART pregnancies versus 1-2% of spontaneous pregnancies. The risk of more than a twin pregnancy is about 3%.

To put these figures into perspective, studies have shown that the rate of successful naturally conceived pregnancy in couples with proven fertility in the past is approximately 20% per month. Therefore, although a figure of 50% may sound low, it is greater than the chance that a fertile couple will conceive in any given cycle.

We advise that patients plan at the onset to make several ART attempts. There is no absolute restriction on the number of times that a couple can attempt ART. Although cumulative pregnancy rates increase through a total of six attempts, the success rate for any given cycle remains constant. A rest period between attempts is recommended. Couples who have achieved an ART pregnancy in the past have an increased likelihood of ART-related conception in the future.

Patient Evaluation

General

Before starting ART therapy, we perform certain tests to ensure that conditions for successful pregnancy are optimal.

You should have a complete physical exam, including breast exam and Pap smear within one year of treatment. You should also start taking prenatal vitamins containing folic acid, which has been shown to reduce the risk of birth defects of the spine. Women over 35 should consider having a mammogram prior to ART therapy.

Blood Tests

We confirm the woman's blood type, and screen for antibodies that could affect the health of a fetus. We also perform a test for syphilis (a venereal disease that can affect the fetus). Documentation of immunity to rubella (German measles) may also require a blood test. Rubella during pregnancy can cause serious harm to the fetus. We require blood tests for hepatitis and HIV (AIDS) for both the patient and her partner. We may recommend a blood test for FSH (a hormone that regulates ovarian function). This must be performed on the second, third or fourth day of the menstrual cycle. This test can reveal abnormalities in ovarian function that can affect the success of ART therapy, especially in women over 35 years of age.

Semen

A semen analysis should be performed within one year of ART. Changes in sperm quality may occur over time, which could affect the success of ART therapy. In some cases, we may recommend additional semen testing. For example, we may test for the presence of anti-sperm antibodies or refer the male partner to urology for a further evaluation.

Uterus

We recommend evaluating the anatomy of the uterus prior to ART. We may suggest an x-ray procedure (hysterosalpingogram, HSG), ultrasound procedure (sonohysterogram, SHG, saline ultrasound), or hysteroscopy. An HSG is performed by injecting a special liquid (X-ray contrast) through the cervix into the uterus. The liquid is visible on x-ray films and outlines the anatomy of the uterus and tubes. This is performed in a radiology suite and requires no anesthesia. An SHG is performed by injecting sterile saline into the uterus during transvaginal sonography. This procedure is performed in the office. Hysteroscopy involves insertion of a small camera through the cervix into the uterus to look for abnormalities.

Prior to IVF/ET, we also perform a mock transfer. The purpose of this procedure is to determine the length and curvature of the uterine cavity. This enables us to guide the embryo transfer catheter into the proper position during the actual embryo transfer. The mock transfer is similar to a pelvic exam or intrauterine insemination. Your physician will place a speculum in the vagina, and insert a thin, flexible plastic catheter through the cervix into the uterus.

ART: A Step-by Step Guide

Every cycle of ART involves multiple steps, and each occurs at a specific time.

Preceding ART Cycle

- Initiation of oral contraceptives
- Initiation of Lupron® or other GnRH analog therapy

ART Cycle

- Ovarian stimulation with gonadotropins (e.g., Gonal-F®, Repronex®)
- Monitoring of follicle development with ultrasound and serum hormone levels
- hCG administration
- Transvaginal oocyte retrieval
- Embryo transfer
- Progesterone supplements
- Hormonal studies and pregnancy test
- Follow-up consultation

Step 1 — Initiation of Oral Contraceptives

We prescribe oral contraceptives prior to the ART cycle. This ensures that GnRH analog therapy will start at the proper time. There is also evidence that oral contraceptives help prevent ovarian cysts, which sometime develop during GnRH analog therapy. You will usually begin a pack of oral contraceptives after your normal period begins. Alternatively, we may prescribe Provera for patients who ovulate irregularly or not at all and do not have regular menstrual periods.

Step 2 - GnRH Analog Administration

You will usually begin treatment with a GnRH analog on or after the sixteenth day of oral contraceptive pills although this may vary. You do not need a pregnancy test before you start the GnRH analog.

We will usually instruct you to reduce the dosage of GnRH analog by one-half on the day you begin ovarian stimulation. You will use the GnRH analog until the day of hCG (human chorionic gonadotropin) administration.

We sometimes treat patients with a different dosage or a GnRH antagonist (Antagon® or Cetrotide®). Your physician will advise you if these changes apply to you.

Step 3 - Baseline Pelvic Ultrasound

Most patients begin a menstrual period 4-10 days after starting GnRH analog therapy. Around the time of your expected period, we will perform an ultrasound to examine the ovaries. If we detect a cyst, we may withhold further therapy until the cysts resolve spontaneously (usually in about a week). Occasionally, we recommend cyst aspiration (drainage). This is a procedure in which your doctor inserts a fine needle connected to a

syringe, guided by ultrasound, into the cyst. We may also perform a serum estradiol measurement to confirm ovarian suppression. Persistent cysts may require cancellation of the cycle.

Step 4 - Ovarian Stimulation

In general, we start ovarian stimulation after menstrual bleeding begins if the baseline ultrasound shows no cysts. We use several similar medications to stimulate follicle (egg) development. Pergonal® and Humegon® are injected intramuscularly (into a large muscle under the skin). Repronex®, Fertinex®, Gonal-F® and Follistim® are injected just under the skin using a smaller needle.

Step 5 - Monitoring of Follicle Development

We monitor follicle development with a combination of vaginal ultrasound and hormone measurements (blood tests). We must perform these tests frequently during the ART cycle to ensure that you take the proper dosage of medication. We usually see patients every other day for an ultrasound and an estradiol level. This allows us to adjust the dose of medication in an effort to improve follicular development. The amount of medication we prescribe each afternoon depends upon the results of the blood tests and ultrasound exams. Typically, the lab results from the blood samples are not available until after 2:00 p.m. Patients must be available by telephone in the afternoon so that we can confirm the dosage of medication for that day.

Step 6 - Final Oocyte Maturation/hCG Administration

Human chorionic gonadotropin (hCG) is a hormonal drug which stimulates the final maturation of the oocytes. Determining the proper day for hCG administration is critical. If it is administered too early, few, if any, oocytes will be mature. If it is administered too late, the eggs within the follicles may be too mature (atretic), and will not fertilize. Optimal oocyte maturity occurs when we administer the hCG at the time two follicles measure at least 16-18 mm and serum estradiol is greater than 500 pg/mL. The drug is given as a single intramuscular injection. Alternatively, Ovidrel® may be given as a single subcutaneous injection. Your physician will let you know which drug you are using and how to give the drug. The time of the injection is based on the time at which we schedule the egg retrieval.

Step 7 - Transvaginal Oocyte Retrieval

Oocyte retrieval is performed 35 hours after hCG has been administered. All retrievals are performed in a dedicate procedure room at Kapiolani Hospital. An anesthesiologist administers intravenous medications (sedatives and pain relievers) in order to minimize the discomfort that may occur during the procedure. Side effects from these medications are much less common than with general anesthesia. Most patients sleep through the procedure but breathe without assistance.

Once you are comfortable and relaxed, your physician will place the ultrasound transducer into the vagina. A guide attached to the transducer leads the needle through the wall of the vagina and into each follicle in the ovaries. Your physician will collect the oocytes and

follicular fluid into a test tube for transport to the embryology lab. The embryologist will examine the oocytes microscopically.

After the retrieval, we will take you to a recovery room. You will be observed for 1-2 hours while the intravenous medications wear off. When you are fully awake, your vital signs are stable, and you have urinated, you will be released to go home. You may have some vaginal spotting and lower abdominal discomfort for several days following this procedure. Generally, patients feel completely recovered within 1-2 days. You should notify us immediately if you develop severe pain, heavy bleeding, or fever after the retrieval.

The number of oocytes we retrieve is related to the number of ovaries present, their accessibility, and the number of follicles that develop in response to stimulation. Ultrasound provides only an approximation of the number of oocytes that one can expect to recover. On the average, 8-15 oocytes are retrieved per patient. More than 95% of retrievals result in the recovery of at least one oocyte.

Step 8 - Insemination of Oocytes

The embryology laboratory staff examines the fluid aspirated from follicles for the presence of oocytes. We routinely aspirate all mature follicles in order to obtain as many oocytes as possible. Not every follicle contains an oocyte, and rarely, a follicle may contain more than one.

It is important to determine the maturity of the oocytes in order to time the insemination properly. The oocyte can only be fertilized during a short interval of about 12-24 hours. If the oocyte is either immature or postmature (too old), it may not be capable of fertilization or normal development. If immature oocytes are obtained at retrieval, they can often mature in the laboratory prior to insemination. Normal pregnancies have occurred with such oocytes.

Semen is usually collected by masturbation the day of the retrieval. The staff will instruct you regarding time of collection. We recognize the pressure that semen collection may generate under these circumstances. In many cases, some flexibility in the timing and even in the method of collection is possible. We may also suggest semen cryopreservation (freezing) before oocyte retrieval.

The laboratory staff prepares the semen specimen for insemination using techniques designed to separate the sperm from other material present in the ejaculate. As a result of this process, we select the most active sperm to inseminate the oocyte. We usually place about 20,000 sperm in a culture dish with each oocyte. The dish is placed into an incubator, which maintains a specific temperature, pH, level of humidity, and concentration of carbon dioxide. After 12-20 hours, the laboratory staff may detect evidence of fertilization under the microscope. In our laboratory, approximately 80% of oocytes fertilize. This figure may be much lower for patients with severe male factor. It is extremely uncommon for couples without male factor infertility to experience complete lack of fertilization.

If the semen analysis has shown a very low sperm count or very poor motility or morphology we may perform intracytoplasmic sperm injection (ICSI) to fertilize the oocyte. In this procedure the embryologist injects one sperm through a very small needle directly into the

oocyte. The fertilization rates using ICSI for severe male factor infertility are nearly identical to the fertilization rates of IVF with normal sperm.

Step 9 - Embryo Transfer

The embryo transfer procedure is performed two to five days after the oocyte retrieval. This procedure is nearly identical to the mock transfer. Your physician will pass the same type of catheter gently through the cervix into the uterus. After waiting for 1-2 minutes to allow any mild cramping to resolve, your doctor will deposit the embryos into the uterine cavity along with an extremely small amount of fluid. You will require no anesthesia for the embryo transfer. You will be discharged after resting for 20 minutes.

Several studies have indicated that maximal IVF-ET pregnancy rates occur in most cases with the transfer of two to three embryos. Therefore, we usually transfer a maximum of three embryos. The transfer of more embryos may increase the likelihood of a multiple pregnancy, which increases the pregnancy risks for the woman and the fetuses. For those cases in which more than three embryos develop, we offer embryo cryopreservation. This allows us to store excess embryos for transfer at a later date. Typically we freeze only good quality embryos that continue to develop five days after the retrieval since these embryos have the best chance of resulting in a pregnancy.

Step 10 - Progesterone Supplements

We will administer progesterone daily beginning on the day of oocyte retrieval. Ordinarily, specialized cells in the follicle will produce progesterone following ovulation. During oocyte retrieval, some of these cells may be removed along with the oocyte. Supplemental progesterone helps prepare the uterine lining for implantation.

This daily medication will continue until your pregnancy test. If the test is positive, you may be advised to continue to take progesterone for several more weeks. This medication historically has been administered as an intramuscular injection, but vaginal administration (suppositories, capsules or Crinone® gel) is now available with equal effectiveness but fewer side effects.

Step 11 - Hormonal Studies and Pregnancy Test

We will usually perform a serum pregnancy test 11-14 days after the embryo transfer. If the test is positive, we will also measure serum progesterone. On occasion, we may repeat tests every two or three days. If the test is negative, we will instruct you to stop the progesterone.

Step 12 - Follow-up Consultation

If the pregnancy test is positive, we will perform a vaginal sonogram about three weeks later. At this point, we are able to identify the number of embryos and can often see a heart beat. The risk of pregnancy loss is low after this developmental milestone. If the ART cycle is unsuccessful, we will schedule an appointment with your physician to review the procedure and discuss future treatment options.

Micromanipulation

Advances in microscopic equipment and knowledge about oocytes, sperm and embryos have lead to the development of new techniques in ART. Micromanipulation refers to the microscopic treatment of individual oocytes, sperm, or embryos in an effort to improve fertilization and/or pregnancy rates. These techniques require specialized equipment and personnel. The most common micromanipulation techniques used currently are intracytoplasmic sperm injection (ICSI), which is used to assist fertilization in cases of severe male factor infertility, and assisted hatching which is used in some cases in an effort to facilitate implantation of the embryos.

Intracytoplasmic Sperm Injection (ICSI)

The ICSI technique has been developed over the past 10 years to treat cases of severe male factor infertility. Candidates for ICSI may include patients with severe reductions in sperm number or motility, regardless of cause and patients with a history of failure of fertilization in conventional *in vitro* fertilization-embryo transfer (IVF-ET). The ICSI technique may also be used to achieve fertilization using surgically extracted sperm from patients with anatomic or surgical conditions (such as vasectomy) which prevent sperm from entering the ejaculate. In all these cases, donor sperm or ICSI may provide the only options for conception.

The ICSI technique attempts to achieve fertilization by the direct injection of a single sperm into the cytoplasm (interior) of the egg. This is accomplished in the following manner: Mature eggs are freed of surrounding cells by a combination of enzyme treatment and microdissection. Using special micromanipulation equipment, the eggs are individually injected with a single sperm. Injected eggs are returned to the laboratory incubator and are treated thereafter as in conventional IVF-ET.

The mechanical placement of a sperm into the egg bypasses all the normal processes of sperm-egg interaction that occur naturally as well as in conventional IVF-ET. These processes normally lead to the selection of the single fertilizing sperm based on its ability to pass through the many layers of cells surrounding the egg, to contact and bind to the egg coating (zona), to penetrate this coating, to contact and merge with the egg cell membrane and ultimately to be drawn into the egg where the genetic material in the sperm joins that of the egg. These interactions help assure that a normal sperm is selected by the egg for fertilization. Even when conventional IVF-ET is performed, the egg is exposed to tens of thousands of sperm from which to choose. In sperm injection, it is the laboratory that chooses. We rely on the size, shape, and motility of sperm to choose the ones for injection. While these characteristics are useful, they do not guarantee that the sperm selected for injection is normal.

The potential consequences of injecting a normal appearing sperm that is in fact abnormal include the development of a genetically abnormal embryo. Previous experience suggests that most abnormal conceptions do not implant or develop in the uterus. The incidence of congenital abnormalities (birth defects) following ICSI appears to be no higher than that of the general population. This observation is based on the experience of several thousand babies born worldwide following ICSI. Recent evidence suggests that some forms of severe male factor infertility are genetic and may be passed on to children through the ICSI procedure. In addition, you must realize that within the normal human population a certain

percentage (approximately 4%) of children are born with physical or mental defects, and that the occurrence of such defects is beyond the control of physicians.

The benefit of ICSI is that it provides a way to treat extreme cases of male factor infertility which otherwise would remain untreatable. Experience shows that fertilization *in vitro* requires a minimum number of motile, normal shaped sperm. The chance for fertilization *in vitro* becomes very low when this minimum number of sperm is not available. The alternatives to ICSI for treatment of severe male factor infertility are limited. Sometimes all the eggs can be placed in one culture dish with all the available sperm. This is known as clutch insemination, which differs from conventional IVF-ET in which each egg is inseminated with a separate batch of sperm. A minimum number of actively moving, normal shaped sperm is still required for fertilization to occur with clutch insemination. Another option is donor sperm. Use of donor sperm normalizes the success of conventional IVF-ET in couples with severe male factor infertility. However, in cases where male factor is the only diagnosis, pregnancies with donor sperm can be achieved through timed intrauterine insemination (IUI), a treatment far less expensive and complicated than IVF-ET.

There is no guarantee that ICSI will result in fertilization or a pregnancy. The likelihood of conception can be decreased by coexisting female fertility problems. In general, the results of intracytoplasmic sperm injection decline with increasing age of the female partner. This probably reflects the progressive decline in oocyte quality with age of the patient and the egg's inability to survive the invasiveness of sperm injection.

An additional charge is required for ICSI.

Assisted Hatching

Normally, embryos are transferred to the uterus three days after retrieval. Usually the embryos consist of eight cells at this stage. After transfer, the embryo must continue to develop to the blastocyst stage (a hollow ball of about 100 cells) before implantation can occur. This development takes several days. Immediately before implantation, the blastocyst must "hatch" from the zona coating which originally enveloped the oocyte. To assist the hatching process, we sometimes micromanipulate the embryos immediately before embryo transfer. This involves either making a slit in the zona using a fine glass needle or dissolving part of the zona coating with an acid solution. This must be performed under the microscope by trained personnel using special micro tools. There is a small risk of damage to the embryos from the procedure. It is not clear which patients are the best candidates for assisted hatching, but we may consider it for patients with repeated unexplained treatment failures or for women 35 years of age or older.

Embryo Cryopreservation

Embryo cryopreservation is another important part of successful ART programs. Cryopreservation affords patients several advantages. Couples can cryopreserve embryos in excess of the ones that are usually transferred during an ART cycle. These embryos provide a second or even third opportunity for pregnancy without undergoing another ovarian stimulation and retrieval.

Those embryos that meet developmental criteria for appearance and rate of growth can be frozen. The freezing process is computer controlled and employs special solutions to protect the fertilized eggs from damage. Frozen embryos are stored at 196°C (or approximately 400°F) below zero. Prior to ART, you and your partner must sign a consent form indicating what we should do with any additional embryos. Current choices are disposal or cryopreservation for your future use. If you choose to store your embryos you must keep us informed of your current address at least annually. Social, ethical, and legal principles related to various aspects of ART have not yet been established. For this reason, you should discuss the implications of cryopreservation with your physician and with an attorney before proceeding with ART. Issues to consider include the disposition of embryos in the event of divorce or the death of either you or your partner. Our current policy is to dispose of cryopreserved embryos if *both* of the original partners to the agreement die.

Many embryos do not survive cryopreservation and thawing. Those that do may function less well than do fresh embryos, that is, they may implant and produce ongoing pregnancies at a somewhat lower rate than fresh embryos. Despite this we have established a very successful frozen embryo transfer (FET) program. In 2003, the nationwide "take home baby rate" per frozen embryo transfer procedure was 27%. Our success rate has been above the national average in recent years.

We will usually transfer *up to* three embryos during this procedure. Embryos can be transferred successfully during either a natural cycle or an artificial cycle in which you take estrogen and progesterone. There is no consensus regarding which approach is "best." Since all of our frozen embryo transfers at Tripler Army Medical Center must be batched in cycles, the natural cycle method is not logistically possible and we use the artificial cycle method.

Frozen Embryo Transfer Using Hormone Replacement: A Step-by-Step Guide

For patients with irregular cycles or ovulation disorders, and for patients who need to plan their therapy around time constraints, we can create an artificial menstrual cycle for FET. This involves treatment with an oral estrogen medication and progesterone (usually administered vaginally). This treatment is well established. Pregnancy rates are equivalent when compared to natural cycle FET. We sometimes recommend a trial (practice) cycle before the actual FET cycle so we can perform an endometrial biopsy to ensure that the medication dosages produce the proper development of the uterine lining. In addition, if you have been pregnant, we recommend a repeat uterine measurement before FET. The steps involved in FET with hormone replacement include:

FET Cycle

1. Initiation of Oral Contraceptives
2. Initiation of Lupron® or other GnRH analog therapy
3. Hormone therapy (Estrace® and progesterone)
4. Embryo transfer
5. Hormonal studies and pregnancy test
6. Follow-up consultation

Step 1 — Initiation of Oral Contraceptives

We prescribe oral contraceptives prior to the ART cycle. This ensures that GnRH analog therapy will start at the proper time. There is also evidence that oral contraceptives help prevent ovarian cysts, which sometime develop during GnRH analog therapy. You will usually begin a pack of oral contraceptives after your normal period begins. Alternatively, we may prescribe Provera for patients who ovulate irregularly or not at all and do not have regular menstrual periods.

Step 2 - GnRH Analog Administration

You will usually begin treatment with a GnRH analog on or after the sixteenth day of oral contraceptive pills although this may vary. You do not need a pregnancy test before you start the GnRH analog.

Step 3 - Estrogen Therapy

It is important that you start estrogen therapy on the first day of your period. A dose of estrogen is usually administered for 14 days, although shorter or longer cycles may be used, Estrace® is the most common form of estrogen we use. This is a pill containing 1 mg of estradiol, the same hormone produced by the ovaries. We will have you take two pills twice a day for about 14 days. After about 14 days of Estrace®, (your physician may vary the dose or duration of therapy) progesterone is added. This may be administered vaginally or as an intramuscular injection. Estrace® and progesterone are continued until the day of the pregnancy test (usually 11-12 days after embryo transfer). If the test is positive, these medications may be continued for several weeks.

Step 4 - Embryo Transfer

Embryo transfer is usually performed on the fifth day of progesterone therapy. As with natural cycle FET, embryos are thawed on the morning of the scheduled frozen embryo transfer. In our laboratory, approximately 80% of embryos survive cryopreservation and thawing. We usually transfer 2-3 embryos during each FET cycle. However, this number is flexible, and we will discuss this issue with you.

The actual embryo transfer itself is identical to the embryo transfer following *in vitro* fertilization-embryo transfer. A small plastic catheter is passed gently through the cervix into the uterus. After waiting for 1-2 minutes to allow any mild cramping to resolve, the embryos are deposited into the cavity along with a small amount of fluid. You will be discharged after resting for 20 minutes. No anesthesia is required for the embryo transfer.

Step 5 - Hormonal Studies - Pregnancy Test

We will usually perform a serum pregnancy test 11-12 days after the embryo transfer. If the test is positive, we also measure serum progesterone. On occasion, we may repeat tests every two or three days. If the test is negative, progesterone is discontinued and a period usually begins in a few days.

Step 6 - Follow-up Consultation

If the pregnancy test is positive, we will perform a vaginal sonogram about three weeks later. At this point, we are usually able to identify the number of embryos and can often see a heart beat. The risk of pregnancy loss is low after this developmental milestone. If the procedure is unsuccessful, we will schedule a consultation with your physician. We will review the procedure and discuss further treatment options.

Donor Oocyte Therapy

In recent years, with the standardization of IVF-ET techniques and the development of ICSI (intracytoplasmic sperm injection) for severe sperm disorders, it has become clear that the single most important factor in predicting the success of IVF-ET is the age of the female partner. For patients under 30, success rates of 30-50% per oocyte retrieval can legitimately be expected; for patients over 40, realistic success rates are only 5% to at most 15%. Oocytes from younger women possess greater fertility potential, and this potential is utilized in donor oocyte therapy. In this therapy, oocytes from another woman (the donor) are fertilized with the patient's (the recipient) husband's sperm, and the resultant embryos are placed in the recipient's uterus. The oocytes are stimulated and retrieved from the donor using routine IVF-ET techniques. The donor may be know to and recruited by the recipient (non-anonymous donation), or instead may be unknown to the recipient, having been recruited by the IVF-ET program (anonymous donation). In cases where a young (less than 35 years old) donor is utilized, high success rates, comparable to those achieved in women of similar age using their own oocytes, can be expected. *We do not offer donor oocyte therapy at Tripler Army Medical Center* but we can refer you to a civilian ART program if you desire this treatment.

Candidates for Donor Oocyte Therapy

There are three main indications for donor oocyte therapy. One is ovarian failure, which can be due to a wide variety of different causes, including radiation, chemotherapy, surgical removal of the ovaries, and a variety of disease states which cause or are associated with ovarian failure. Another indication is for women who carry some serious genetic disease who wish to diminish the chances that the disease will be passed on to their offspring. The third, and most common indication, is for women whose age is sufficiently advanced that their fertility potential is impaired significantly.

ART Medications

GnRH Analogs

Gonadotropin releasing hormone (GnRH) is a hormone produced in the brain which indirectly stimulates ovarian function. Analogs of GnRH are synthetic forms of this hormone which do not directly induce follicle development or ovulation but which have become very important in ART therapy. There are several advantages to using GnRH analogs. First, they make ovarian stimulation easier to regulate, since the patient's own hormone production is suppressed. Second, patients who are treated with GnRH analogs tend to produce a greater proportion of mature oocytes than patients who do not receive them. Third, GnRH analogs markedly decrease the risk of cycle cancellation for most patients. Prior to their use, 20-30% of IVF-ET cycles were canceled because patients would have a premature LH surge with spontaneous ovulation. Using GnRH analogs, the risk of cycle cancellation is less than 5%. Fourth, ovarian function can be suspended with GnRH analogs for variable periods of time if necessary, which allows for flexibility in cycle scheduling.

The major disadvantage of GnRH analogs is that most patients require more medication for ovarian stimulation. This increases the cost of an ART cycle. Occasionally, patients require adjustments in dosage of GnRH analogs, or may respond better to treatment without analogs. Your doctor can discuss these issues with you.

Mechanism of Action

Analogs of GnRH initially stimulate the pituitary gland to release all the stored gonadotropins (LH and FSH - the hormones that normally stimulate ovarian function). Over the course of a week to ten days, GnRH analogs suppress the production of any new LH and FSH. This effect appears to prevent the ovaries from receiving mixed signals - from the patient's own LH and FSH and from the medications that we administer to stimulate follicle development. The result for many patients is a more synchronized development of mature oocytes.

Dosage and Monitoring

The GnRH analog we use most commonly is leuprolide acetate (Lupron®). Lupron® must be injected to be active. In ART therapy, we use a formulation of Lupron® which can be injected just under the skin, in a manner similar to insulin injections for diabetes therapy.

The usual dosage of Lupron® is 0.1 or 0.2 cc daily as a single injection. Menstruation usually occurs four to ten days later. During the time of actual ovarian stimulation, the dosage of Lupron® is halved (e.g., 0.1 cc to 0.05 cc daily). Lupron® is usually administered until the day of hCG administration. Some patients, because of their history or condition, are treated with a different dosage or schedule of Lupron®. Your physician will advise you if these changes apply to you.

Adverse Effects

Adverse effects from GnRH analogs are uncommon. Occasionally, ovarian cysts may form during therapy. These usually resolve spontaneously. Rarely, cysts may grow so large as to cause abdominal bloating and pain. Even less common is ovarian torsion, in which the ovary

twists and cuts off its own blood supply. Surgical removal of the ovary may be necessary in these very rare circumstances.

Other adverse effects of GnRH analogs include headaches, mood changes, and altered sleep. Hot flashes may occur during prolonged therapy. Allergic reactions are rare. A slight redness and discomfort may occur at the Lupron® injection site.

GnRH Antagonists

Some patients may not be treated with GnRH analogs. To prevent premature ovulation and cancellation of the cycle in these patients a GnRH antagonist is used. The antagonists also block the secretion of LH, but do not require prolonged treatment. Therefore they can be given after stimulation of the ovary has started which reduces the amount of medication required. Some patients, especially older patients or patients with decreased ovarian function, may respond better and produce more oocytes using these drugs.

Mechanism of Action

Antagonists of GnRH suppress the production of any new LH and FSH without the initial stimulation which occurs with GnRH analogs. This allows the patient's own FSH to be produced early in the stimulation cycle prior to receiving the GnRH antagonist. The result for some patients is a better stimulation and development of mature oocytes.

Dosage and Monitoring

The GnRH antagonist we use most commonly is ganirelix acetate (Antagon®) although we may also use cetrorelix acetate (Cetrotide®). Both must be injected just under the skin to be active. The usual dosage of Antagon® is 250 µg daily. The dosage of Cetrotide is either 0.25 mg daily or 3 mg every three days. These drugs are usually started during the time of ovarian stimulation when the biggest follicle is 12 – 14 mm in diameter and continued until the day of hCG administration.

Adverse Effects

Adverse effects from GnRH antagonists are uncommon. Mild and short lasting reactions may occur at the injection site like reddening, itching, and swelling. Abdominal pain, nausea and headache are also possible side effects

Gonadotropins

To increase the likelihood of pregnancy through ART, multiple oocytes must be produced. This is accomplished through the administration of gonadotropins-hormonal medications which stimulate the ovaries. Stimulation can be achieved with a variety of drug regimens. Gonadotropin medications come in several forms. Pergonal®, Repronex® and Humegon® are combinations of FSH and LH. They replace a woman's own LH and FSH which are normally produced by the pituitary gland. Fertinex®, Gonal-F® and Follistim® are preparations that contain only FSH. Gonal-F® and Follistim® are recombinant products which are made by genetically engineered cells. This process ensures uniform purity and potency. Because the dose of hormones we use in ART is greater than what the body

normally produces, the ovaries typically develop more than one oocyte as occurs in a natural cycle.

Gonadotropins act directly on the ovary to stimulate the growth of follicles (the structures in ovaries which contain eggs). These developing follicles can be counted and measured using transvaginal ultrasound. As the follicles grow, they produce increasing amounts of estrogen, which can be measured with a laboratory blood test.

Dosage and Monitoring

Gonadotropins are packaged in vials containing 75 or 150 International Units (IU). In the first cycle of IVF-ET we routinely administer 300 - 450 IU of gonadotropins daily for three days. This dosage may vary depending on the patient's history. We then see patients in the office for regularly scheduled transvaginal ultrasound examinations and serum estradiol tests. The dose of gonadotropins is then determined by the result of the ultrasound and estradiol tests. Most women require between seven to ten days of gonadotropin therapy.

Pergonal® and Humegon® require intramuscular injection, usually into the muscles of the buttocks. Fertinex®, Repronex®, Gonal-F® and Follistim® are administered subcutaneously, like an insulin or allergy shot.

Adverse Effects

Gonadotropin preparations are strong medications. Although rare, a potentially serious adverse effect of gonadotropins is ovarian hyperstimulation. Even after oocyte retrieval, the ovarian tissue may continue to grow in response to the prior gonadotropin stimulation. As the ovaries enlarge, discomfort and bloating may occur. Occasionally, an enlarged ovary may become twisted. This condition is referred to as ovarian torsion. When this occurs, surgery may be required to either remove the ovary or untwist it.

In addition to discomfort, women suffering from severe ovarian hyperstimulation may develop ascites (a collection of fluid in the abdomen or pelvis). This fluid enters the pelvis by leaking through blood vessels. Although rare, this condition can be severe enough to produce swelling of the abdomen and shortness of breath. Hospitalization is required in cases of severe ovarian hyperstimulation. Treatment for ovarian hyperstimulation usually consists of bed rest and intravenous fluids. On rare occasions it is necessary to drain fluid from a patient's abdomen. Hyperstimulation is more severe when pregnancy occurs, as the developing pregnancy produces the hormone hCG, which stimulates the ovaries to continue to grow. Hyperstimulation can remain a potential problem for to 2-3 months during the pregnancy.

There does not appear to be any increased risk of birth defects in offspring of women who take gonadotropins compared to conceptions in the general population. However, there is a greater risk of early miscarriage in patients taking gonadotropins. Approximately 20-25% of gonadotropin induced conceptions miscarry within the first trimester. Multiple pregnancy is another adverse effect of gonadotropins therapy. Approximately 33% of IVF-ET pregnancies are multiple. The risk of more than twins is about 3%.

Although not truly an "adverse effect," the cost of gonadotropins must be taken seriously. One ampule (amp) of 75 IU typically costs about $50 to $70. As these medications are commonly administered for seven to ten days, it is not unusual for the medication cost for a single cycle to cost $1000 to $2000.

In summary, gonadotropins are strong, effective medications for inducing follicle development. Their use must be monitored carefully, preferably with a combination of regular transvaginal ultrasound examinations and estradiol determinations. When administered and monitored carefully, the risk of adverse effects is acceptably low.

Human Chorionic Gonadotropin

Human chorionic gonadotropin (hCG) is an injectable medication that is administered to complete oocyte maturation. The brand names for hCG are Ovidrel®, Profasi® and Pregnyl®. Ovidrel is a recombinant product which is made by genetically engineered cells and it is injected subcutaneously. Profasi® and Pregnyl® are injected in the muscle.

Mechanism of Action

Human chorionic gonadotropin is structurally similar to the LH which is produced by a woman's pituitary gland. It acts on the ovary in a manner similar to a woman's own LH. Human chorionic gonadotropin, like LH stimulates the final maturation of the oocytes in the follicle. It also stimulates progesterone production from the ovary after egg retrieval. This progesterone is important to prepare the uterus for implantation of the embryo.

Dosage and Administration

Once hCG is administered, ovulation usually occurs in approximately 36 to 40 hours. We therefore routinely schedule oocyte retrieval at 35 hours after hCG. This helps ensure maximal egg maturity, which is important for fertilization and embryo development.

The dose of Ovidrel® is 250 µg. It is injected subcutaneously as a single dose. Profasi® and Pregnyl® can be administered several different ways. We commonly administer a single injection of 10,000 units. Occasionally, several doses of 2,500 units (usually every three days) are administered after egg retrieval to stimulate progesterone production.

It typically takes 8-10 days for single injection of hCG to be cleared from the blood stream. As hCG is the same hormone that is produced by a developing pregnancy, patients should not have a blood or urine pregnancy test sooner than ten days following the hCG injection. If a pregnancy test is performed earlier, it may measure the hCG that was given by injection rather than measure hCG produced by a pregnancy.

Adverse Effects

When given by itself, there are few, if any adverse effects to hCG. However, when given in conjunction with gonadotropins, ovarian hyperstimulation can occur.

Medication Injection Instructions

Lupron®

1. Wash and dry hands thoroughly.
2. Assemble supplies: two alcohol wipes, one tissue, one syringe, medication.
3. At first use, remove and discard plastic cap off the medication.
4. Clean the Lupron vial with an alcohol wipe.
5. Remove cap from syringe exposing needle.
6. Pull the syringe plunger back until its tip is at the proper dose mark. Insert needle straight and firmly into the rubber center of the vial and push the plunger all the way in.
7. Turn the vial upside down.
8. Pull the syringe plunger down filling the medication slightly below the line (10 or 5 units depending on your dose) and remove the needle from the vial.
9. Hold the syringe needle up and flick lightly with finger to remove any air bubbles.
10. Hold the syringe and new alcohol wipe in the right hand.
11. Choose injection site (stomach or thighs), rotating daily. Pinch the skin gently with the left hand.
12. Wipe area then save wipe to wipe the area again after the injection.
13. Holding the syringe like a dart, perpendicular to the skin, briskly insert small needle quickly and entirely into the skin.
14. Slowly inject all medication, release the pinch and remove syringe. Cover the area with alcohol wipe then with tissue.
15. When you begin gonadotropin injections, the Lupron dosage will usually decrease to half (5 units) or be discontinued. Medication may continue through the day of hCG administration or may be terminated when you begin gonadotropin injections.

NOTE:

1. Lupron should be stored in the refrigerator or in a cool, dry environment away from heat and humidity.
2. A small amount of blood at the injection site is NORMAL. Applying pressure to the site (after administering the shot) will stop the bleeding.
3. Bruising may occur. Although this is normal, it is recommended that you alternate sites.
4. Lupron once a day = administer in the PM between 6pm – 10pm
5. Lupron twice a day = between 6am - 10am & 6pm - 10pm
6. NO Lupron after the hCG Injection.

Gonal-f®

See also the following web site for a video demonstration:

http://www.fertilitylifelines.com/serono/products/gonalf/vial/instructions.jsp

Repronex®

Equipment:

- Medication (powder)
- 3 cc 22 g 1 1/2" syringe
- 27 g 1/2" needle
- Diluent (saline)
- Alcohol swab
- Gauze pad or tissue
- Rubbing alcohol

Note: Your injection materials MUST be sterile and MAY NOT be reused

To prepare medication:

1. Remove the syringe from the package.
2. Flip off the seals from the top of the medication and the diluent.
3. Wipe the rubber stoppers with rubbing alcohol.
4. Remove the cap from the syringe.
5. Draw air into the syringe by pulling the plunger out to the 1 cc (l ml) mark. Inject the 1 cc air into the vial of diluent (saline).
6. Push the syringe to the bottom of the vial. Pull back on the plunger to fill the syringe with 1 cc (l ml) of diluent.
7. Slowly inject all the diluent from the syringe into the vial of medication (powder). The medication will dissolve readily. Swirl gently (do not shake).
8. Push the syringe to the bottom of the vial and withdraw the entire 1cc of dissolved medication. For doses greater than 1 vial, take the dissolved medication and inject it into the next vial of powder until the correct dose is reached. Up to 4 powders can be mixed in 1 cc of diluent.

Administration:

1. Twist off the needle tip of the syringe and replace with a new 27 g 1/2" needle tip. Make sure the new needle tip is twisted securely in place.
2. Choose the injection site: back of upper arms, abdomen or outer top of thigh.
3. Clean the injection site with rubbing alcohol and let dry.
4. Pinch the injection site with your non-dominant hand.
5. Use your dominant hand to insert the needle at a 45-degree angle with a quick, dart-like motion. Slowly, steadily depress the plunger to inject the medication.
6. Withdraw the needle and discard the syringe. Use a gauze pad or tissue to apply pressure at the injection site.

Note: SQ administration of Repronex may cause a raised red area at the injection site that should improve within 24 hours.

Ganirelex acetate (Antagon®)

Antagon is used to prevent ovulation during ovarian stimulation. This medication must be taken every 24 hours until a human Chorionic Gonadotropin (hCG) such as Pregnyl or Ovidrel is given. You will begin taking this medication as instructed, when follicles measure 12 to 14 mm.

Equipment:

- Prefilled Antagon syringe
- Alcohol swabs
- Tissue or gauze pad
- Rubbing alcohol

Administration instructions:

1. Choose injection site: back of upper arms, abdomen or anterior thigh.
2. Clean injection site with rubbing alcohol and let dry.
3. Pinch the injection site with your non-dominant hand.
4. Using your dominant hand, hold the syringe like a pencil, and with a quick, dart-like motion, insert the syringe at a 90-degree angle.
5. Slowly, steadily depress the plunger until all the medication is injected. The medication may burn as you depress the plunger; this is normal.
6. Withdraw the needle and discard the syringe.
7. Place a gauze pad or tissue over the injection site. If any bleeding occurs, apply pressure to the site.

See also the following web site for a video demonstration:

http://www.follistim.com/Consumer/Ganirelix/HowtoAdminister/index.asp

Cetrotide®

1. Wash your hands thoroughly with soap and water.

2. On a clean flat surface, lay out everything you need (one vial of powder, one pre-filled syringe, one injection needle with a yellow mark, one injection needle with a grey mark, and two alcohol wipes).

3. Flip off the plastic cover of the vial. Wipe the aluminum ring and the rubber stopper with an alcohol wipe.

4. Take the injection needle with the yellow mark and remove the wrapping. Take the pre-filled syringe and remove the cover. Twist the needle on the syringe and remove the cover of the needle.

7. Draw the total contents of the vial into the syringe. If liquid is left in the vial, invert the vial, pull back the needle until the opening of the needle is just inside the stopper. If you look from the side through the gap in the stopper, you can control the movement of the needle and the liquid. It is important to withdraw the entire contents of the vial.

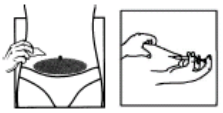
8. Detach the syringe from the needle and lay down the syringe. Take the injection needle with the grey mark and remove its wrapping. Twist the needle on the syringe and remove the cover of the needle.

5. Push the needle through the center of the rubber stopper of the vial. Inject the water into the vial by slowly pushing down on the plunger of the syringe.

6. Leave the syringe in the vial. Gently shake the vial until the solution is clear and without residue. Avoid forming bubbles during dissolution.

9. Invert the syringe and push the plunger until all air bubbles have been pushed out. Do not touch the needle or allow the needle to touch any surface.

10. Choose an injection site in the lower abdominal area, preferably around, but at least one inch away from the belly button. If you are on a multiple dose (0.25 mg) regimen, choose a different injection site each day to minimize local irritation. Take the second alcohol wipe and clean the skin at the injection site and allow alcohol to dry. Inject the prescribed dose as directed by your doctor, nurse or pharmacist.



11. Use the syringe and needles only once. Dispose of the syringe and needles immediately after use (put the covers on the needles to avoid injury). A medical waste container should be used for disposal.

See also the following web site for a video demonstration:

http://www.fertilitylifelines.com/serono/products/cetrotide/instructions/0.25mg.jsp

Ovidrel®

Administration of Ovidrel® PreFilled Syringe

Get ready

1. Make sure you have all the necessary materials assembled in a clean area: Ovidrel® PreFilled Syringe, alcohol swabs, gauze, sharps-disposable container.

Please allow the pre-filled syringe to adjust to room temperature before you administer your injection.

2. Wash your hands thoroughly.

Prepare your Ovidrel® PreFilled Syringe

3. With the needle pointing upwards, carefully remove the needle cap from the syringe. Do not touch the needle or allow it to touch any surface. Keep materials sterile.

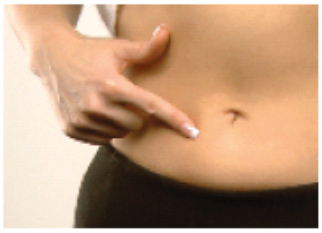
4. To remove any air bubbles, point the needle up and gently tap on the syringe until all the bubbles rise to the top.

5. Push the plunger carefully until a small drop of liquid begins to appear from the tip of the needle.

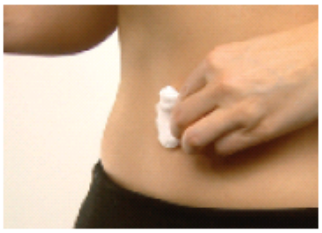
Ovidrel® (Continued)

Prepare the injection area

6. Choose an injection site in the lower abdominal area, preferably around the belly button but at least 1 inch away.



7. Carefully clean the injection site on the stomach with an alcohol swab and allow it to air-dry.



Administer your injection

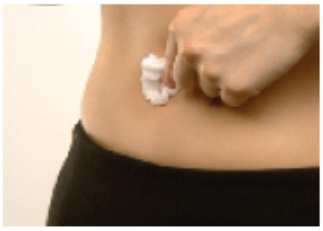
8. Holding the syringe with one hand the way you would hold a pencil, pinch the skin on the chosen injection site with the other hand and hold firmly.

9. Insert the entire length of the needle into the skin at an upward angle of about 45 to 90 degrees, as indicated by your doctor or nurse.

10. Release the skin and push the plunger in a slow, steady motion until all the medication is injected. Take as much time as you need to inject all the contents.

11. After injecting all the contents, gently withdraw the needle.

12. Apply pressure to the injection site with a gauze pad. If bleeding does not stop within a few minutes, place a piece of clean gauze over the injection site and cover it with an adhesive bandage.



Discard used materials

13. Discard the syringe in your sharps-disposal container. Remember that injection materials must be kept sterile and cannot be reused.

Always take your injection exactly as your doctor has instructed.

Storage of Ovidrel® PreFilled Syringe

Important: Ovidrel® PreFilled Syringe should be stored refrigerated (36°- 46°F/ 2-8°C) to allow the product to be used until the expiry date shown on the syringe or carton. Alternatively, the Ovidrel® PreFilled Syringe may be stored by the patient for no more than 30 days at room temperature (up to 77°F / 25°C) and in this case must be used within those 30 days. Protect from light. Do not freeze.

See also the following web site for a video demonstration:

http://www.fertilitylifelines.com/serono/products/ovidrel/instructions.jsp

hCG (Pregnyl® and Profasi®)

Equipment:

- Medication (powder)
- Diluent (sterile water)
- 3 cc 22 g 1 1/2” syringe
- 22g 1 1/2” needle
- Alcohol swab
- Rubbing alcohol

To prepare medication:

- Using your thumb, flip off the plastic lids to reveal the rubber stoppers on each vial.
- Push the syringe through the rubber stopper to the bottom of the diluent (sterile water) vial. Pull back on the plunger to withdraw 1 cc (1 ml) of diluent. There will be a large amount of sterile water remaining that you may discard.
- Slowly inject the 1cc of diluent from the syringe into the vial of medication. Shake the vial gently until the powder dissolves completely.
- Push the syringe to the bottom of the vial and pull back on the plunger until all the dissolved medication is in the syringe.
- Remove the syringe from the vial.

hCG administration:

- Twist off the needle tip of the syringe and replace with a new 22 g 1 1/2” needle tip. Make sure the new needle tip is twisted securely in place.
- Choose injection site: upper outer quadrant of the buttock, right or lift side.
- Clean injection site with rubbing alcohol and let dry. (You may numb the area with an ice cube before cleaning the site.)
- Lie on your side with your knee slightly bent.

THE FOLLOWING STEPS SHOULD BE COMPLETED BY YOUR PARTNER:

- Use the thumb and first two fingers of your non-dominant hand to hold the skin in position.
- Using your dominant hand, hold syringe like a pencil and with a quick, dart-like motion, insert entire needle at a 90-degree angle. Once the needle is in place, release your grasp of the skin.
- Slowly pull back on the plunger with your non-dominant hand (the hand that just let go of the skin). If blood enters the syringe at this time, pull the needle out of the skin slightly and test again.
- If no blood appears, depress plunger in a slow, steady motion until all medication is injected.
- Withdraw needle and discard the syringe. Use gauze pad or tissue to apply pressure at the injection site.

Note: You may notice some soreness at the injection site the next day (similar to the feeling of a sore muscle). Occasionally, some women experience a brief period of nausea.

Progesterone

Equipment:

- Medication
- 3 cc 22 g 1 1/2" syringe
- Needle
- Alcohol swab
- Rubbing alcohol

Note: Your injection materials MUST be sterile and MAY NOT be reused

To prepare medication:

1. Remove the syringe from the wrapper.
2. Remove the needle cap. Pull back on the plunger of the syringe, filling it with the same amount of air as the volume of medication to be administered. For example, if 1 cc of medication is desired, fill syringe with 1 cc of air.)
3. Cleanse the rubber stopper with an alcohol swab.
4. Insert the needle through the rubber stopper and depress the plunger, forcing the air into the vial.
5. Turn the vial upside down and pull back on the plunger, allowing the desired amount of medication to enter the syringe.
6. Withdraw the needle from the vial.

To administer medication:

1. Twist off the needle tip of the syringe and replace with a new 22 g 1 1/2” needle tip. Make sure the new needle tip is twisted securely in place.
2. Choose injection site: the upper outer quadrant of the buttock, left or right side.
3. Clean the injection site with rubbing alcohol and let dry.
4. Lie on your side with your knee slightly bent
5. Hold the syringe as you would a pencil and with a quick dart-like motion, insert the entire needle at a 90-degree angle.
6. Pull back on the plunger to check for proper placement.
7. Slowly, steadily depress plunger until all the medication is injected.
8. Withdraw the needle and discard the syringe. Use gauze or tissue to apply pressure after the injection.

Note: Progesterone can sometimes cause lumps or tenderness at the injection site. This may be relieved by messaging the area for a couple of minutes after the injection or applying heat to the site.

Oocyte Retrieval Instructions

Day of hCG injection:

1. Go to the pharmacy and pick up the medications for after the oocyte retrieval. The medicines are: Progesterone, doxycycline, Tylenol, Roxicet, colace and medrol. Some patients will also receive estrace.
2. Take the Ovidrel or hCG at the time instructed according to the instructions in this manual. It is very important to take the medicine at the specified time. If you do not take this medication within 30 minutes of the instructed time let us know immediately.
3. Stop the lupron and gonadotropins.
4. Continue taking the prenatal vitamins.
5. Husband should have an ejaculation today.

Day of oocyte retrieval:

1. Do not eat or drink anything after midnight prior to the retrieval.
2. Do not wear perfume or scented deodorant to the retrieval.
3. Do not wear jewelry or contact lenses.
4. Dress comfortably.
5. The retrieval will be at Kapiolani. Please be there at the time instructed.
6. Arrange to have someone available to drive you if your husband will not be present. You will not be able or permitted to drive yourself home. Limit your activity for 24 hours after the retrieval.
7. The embryologist will contact the husband immediately after the oocyte retrieval with instructions for obtaining the semen sample. In some cases such as with donor sperm, frozen sperm or surgically retrieved sperm you will be given special instructions.
8. Expect some spotting and discomfort following the procedure. Take the Tylenol or roxicet if needed for pain control. Do not take aspirin, motrin, ibuprofen, aleve or any other pain medications.
9. If you have fevers, heavy bleeding or pain not controlled with the medications call us immediately at 433-5496 during duty hours or page 577-5534 during non duty hours.

Medications:

1. *Doxycycline*. You will be given 10 tablets. Take one tablet the morning of the retrieval with a sip of water only. Take one tablet twice a day with food beginning the evening of the retrieval.
2. *Medrol* (aka methylprednisolone). You will be given 16 tablets. Take two tablets twice a day (total of four tablets per day) for 7 days starting the night of the retrieval.
3. *Progesterone*. The progesterone comes in several forms. Most of you will receive either intramuscular injections or vaginal suppositories. Begin taking the progesterone as instructed the night of the retrieval.
4. *Colace*. Take one tablet a day for two weeks if needed to prevent constipation.
5. Take any other medications as instructed.

Embryo Transfer Instructions

1. We will call you the day after the retrieval to give you the fertilization results.
2. The embryo transfer will be on the 2nd, 3rd or 5th day after the retrieval. The day of the transfer depends on your age, the number of embryos and the quality of the embryos.
3. Do not wear perfume or scented deodorant to the transfer.
4. Come to the transfer with a full bladder (but not uncomfortably full.)
5. Limit your activity for 24 hours after the transfer. After 24 hours, you may return to your normal activity but avoid strenuous exercise and heavy lifting. You may shower, but do not take baths or go swimming until after your pregnancy test. Refrain from intercourse until your pregnancy test.
6. Since you may be pregnant do not drink alcohol, smoke or take any medications not approved by one of the physicians.
7. Weigh yourself every day and record the weights.
8. Keep yourself well hydrated with Gatorade or juices.
9. Continue your medications as instructed.
10. Do not stop the progesterone until told to do so.
11. Come to Tripler for a pregnancy test on the day instructed. Do not take home pregnancy tests prior to this since they may be falsely positive due to the hCG injection.
12. If you have fever, bleeding, pain or shortness of breath call 433-5946 during business hours. After duty hours page the resident on call at 577-5534. If you have a true emergency after duty hours go to the emergency department at Tripler.

General ART Questions and Answers

Q: Does ART damage the ovaries?

A: There is no evidence to suggest that laparoscopy and/or oocyte retrieval damage the ovaries. There is one report which suggests that infertile women who take fertility drugs and do not get pregnant have an increased risk of ovarian cancer. However, the study did not collect information on the type of drugs used, and the control (comparison) population may not have been selected accurately. The fertility drugs used in ART have been in use over 30 years, and other studies have suggested no increased risk.

Q: Why is the success rate with ART so low?

A: Studies of human reproduction indicate that for a couple with proven fertility, the likelihood of conception is only 20% per month. ART affords couples with infertility higher chances for conception.

Q: We're concerned about multiple births from ART. Should we just have one embryo transferred?

A: Any time more than one embryo is transferred, the chance for multiple pregnancy exists. In fact, about 30% of births from ART are twins, a rate much greater than in the general population (1 in 80 pregnancies). Triplets and quadruplets have also been conceived through ART. However, the majority of ART pregnancies are singletons, and the chance of *any* pregnancy with ART increases with the number of embryos or oocytes transferred. Success rates appear to peak on average with transfer of two or three embryos. We will discuss the options and implications of transferring fewer than two embryos, but in general we will not recommend transferring just one. Although we do not directly offer it, selective reduction is available to couples who conceive multiple gestations. We can provide you with more information about this procedure.

Q: Is there an increased chance of birth defects if I become pregnant through ART?

A: No. The risk of congenital anomalies in children conceived through ART is the same as the risk in the general population. Chromosome abnormalities, such as Down syndrome also occur at a rate similar to the general population.

Q: I had my tubes tied (tubal ligation) several years ago. Would I be a candidate for IVF?

A: Although surgical reversal of tubal sterilization may be a better option, IVF-ET is still a consideration, especially in older women or in couples with male factor infertility. The success rate is greater for ligation reversal than for a single cycle of IVF-ET, although the results of IVF-ET are obtained more rapidly than ligation reversal. If ligation reversal has been attempted and has failed, IVF-ET represents the best option. Cost and other factors involved in surgical reversal must be considered when making this decision.

Q: How many days does the entire procedure take?

A: The entire procedure takes approximately six weeks. However, we only need to see you intensively at Tripler Army Medical Center over a two-week period. These details are discussed in the "Step by Step" sections.

Q: Can we have intercourse while attempting ART?

A: Yes. We recommend that the man abstain from ejaculating for at least 48 hours preceding egg retrieval. This precaution assures that the semen sample for ART is of optimal quality. Near the time of egg retrieval, the ovaries can be enlarged and tender, which can make intercourse uncomfortable. We recommend that you abstain from intercourse following the retrieval until the pregnancy test.

Q: What if I ovulate before the retrieval?

A: Virtually all cases of premature ovulation are now prevented by the use of GnRH analogs or GnRH antagonists. In rare cases in which these medications are not used, we perform an ultrasound prior to retrieval to make sure the follicles are intact. In the uncommon case of ovulation, we will not perform retrieval because the quality of the remaining oocytes is affected adversely.

Q: Will scar tissue around my ovaries make it impossible to retrieve oocytes?

A: No, the oocyte can usually be retrieved by transvaginal aspiration even when the ovaries are covered with scar tissue. In rare cases, scarring pulls the ovaries out of the normal pelvic position. This condition can be identified before ART with ultrasound.

Q: How much activity is recommended after ET?

A: We recommend a fairly quiet 24 hours after ET. Thereafter, most patients resume their normal routines. Strenuous exercises, running, intercourse, etc. should be avoided until a pregnancy test has been performed.

Q: After embryo transfer, how long must we wait until we have intercourse without risk to the embryo?

A: No one knows for sure. We recommend abstinence until the pregnancy test.

ART Financial Information

A cost estimate for an ART cycle is difficult to provide because some costs vary considerably between patients. Because the ART procedures involve multiple steps, any patient who does not proceed to a further step is usually charged only for the cost of the completed procedures.

The costs for embryology procedures by Pacific IVF at Kapiolani Medical Center are shown below. We have no control over the fees and all inquiries regarding fees and payments must be directed to Pacific IVF. There is no payment plan and payment is required 6 weeks prior to starting IVF.

Procedure	**Cost**
Basic in vitro fertilization (includes semen analysis and assisted hatching if required)	$4,070
Cryopreservation of embryos (includes first year of storage)	$681
Storage of embryos per month (beyond first year)	$42
Frozen embryo transfer	$1,250
Intacytoplasmic sperm injection (ICSI)	$1,250
Cancellation prior to retrieval	$260
Cancellation prior to embryo transfer	$2,083

Additional expenses to consider may include loss of wages from time missed at work, as well as, expenses incurred by travel and accommodations for our out-of-town patients. All of these factors must be considered in ascertaining the financial feasibility of participation in an ART program.

We are sensitive to the tremendous financial investment that couples make to participate in this program. We continuously strive to keep our costs manageable and to maximize the chance of a successful pregnancy.

Additional Resources

Literature:

Infertility-Medical

Corson, Stephen L., M.D. *Conquering Infertility: A Guide for Couples*. New York: Prentice Hall Press, 1990.

Franklin, Robert R., M.D. and Dorothy Kay Brockman. *In Pursuit of Fertility: A Consultation With a Specialist*. New York: Henry Holt & Co., 1990.

Nachtigall, Robert, M.D. and Elizabeth Mehran. *Overcoming Infertility*. New York: Doubleday, 1991.

Novotny, Pamela Patrick. *What You Can Do About Infertility*. New York: Dell Publishing, 1991.

Karow, William G., M.D., William C. Gentry, D.H.D., Christopher Hsuing, and Andrienne Pope, Ph.D. *A Baby of Your Own: New Ways to Overcome Infertility*. Dallas, Texas: Taylor Publishing Co., 1992.

Robin, Peggy. *How to be a Successful Fertility Patient*. New York: William Morrow and Co., Inc., 1993.

Infertility-Emotional

Becker, Gay. *Healing the Infertility Family*. New York: Bantam Books, 1990.

Hill, Susan. *One Woman's Passionate Quest to Complete Her Family*. New York: Viking Penguin, 1990.

Mullens, Anne. *Missed Conceptions: Overcoming Infertility*. Toronto, Canada: McGraw-Hill Ryerson, 1990.

Clapp, Diane, BSN, RN and Merle Bombardieri, LICSW. *How Can I Help? A Handbook for Family and Friends of Couples Going Through Infertility*. Fertility Counseling Associates, 33 Bedford St., Lexington, MA 02173, 1991.

McGuirk, James and Mary Elizabeth McGuirk. *For Want of a Child: A Psychologist and His Wife Explore the Emotional Effects and Challenges of Infertility*. New York: Continuum, 1991.

Salzer, Linda P. *Surviving Infertility: A Compassionate Guide Through the Emotional Crisis of Infertility*. New York: Harper Perennial, 1991.

Borysenko, Joan. *Minding the Body, Mending the Mind*. New York: Bantam Books, 1987.

Stephenson, Lynda Rutledge. *Give Us a Child: Coping with the Personal Crisis of Infertility*. New York: Harper and Row, 1987.

Baughan, Jill. *A Hope Deferred: A Couple's Guide to Coping with Infertility*. Oregon: Multnomah Press, 1989.

Assisted Reproductive Technologies

Centers for Disease Control and Prevention. *2003 Assisted Reproductive Technology Success Rates*. U.S. Department of Health and Human Services, 2003.

Wisot, Arthur L., M.D. and David R. Meldrum, M.D. *A Guide to In Vitro Fertilization and Other Assisted Reproductive Methods*. New York: Parohs Books, 1990.

Silber, Sherman, J., M.D. *How to Get Pregnant With the New Technology*. New York: Warner Books, Inc., 1991.

Lauritzen, Paul. Pursuing Parenthood-Ethical Issues in Assisted Reproduction. Bloomington: Indiana University Press, 1993.

Partridge-Brown, Mary. *In Vitro Fertilization Clinics - A North American Directory of Programs and Services*. McFarland and Company, 1993.

Sher, Geoffrey, M.D., and Virginia Marriage. *From Infertility to In Vitro Fertilization*. New York: McGraw Hill, 1988.

Organizations:

RESOLVE
National Headquarters:
1310 Broadway
Somerville, MA 02144-1731
(617) 623-1156
(617) 623-0744 - Telephone Helpline
www.resolve.org

RESOLVE of Hawaii
(808) 528-8559
www.resolveofhawaii.org
e-mail: info@resolveofhawaii.org

The Endometriosis Association
P.O. Box 92187
Milwaukee, WI 53202
(414) 355-2200
(800) 992-3636
www.endometriosisassn.org

The American Society for Reproductive Medicine
1209 Montgomery Highway
Birmingham, AL 35216
(205) 978-5000
www.asrm.com

The North American Council on Adoptable Children
1821 University Ave., Suite N498
St. Paul, MN 55104
(612) 644-3036
www.cyfc.umn.edu/adoptinfo/nacac.html

Centers for Disease Control and Prevention
1600 Clifton Road NE
Atlanta, GA 30333
(404) 639-3311
www.cdc.gov

Patient Information Sheets

From The American Society of Reproductive Medicine